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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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PRIORITY DOCUMENT

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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NEW CONSTITUTIVE PLANT PROMOTERS

FIELD OF THE INVENTION

The invention is directed to new constitutive plant promoters, more specifically those promoters which can be produced by assembling parts of promoters which have a complementary specificity.

BACKGROUND ART

Genetic engineering of plants has become possible by virtue of two discoveries: first of all the possibility of transformation of heterologous genetic material to the plant cell (most efficiently done by the bacterium *Agrobacterium tumefaciens* or related strains) and secondly by the existence of plant promoters which are able to drive the expression of said heterologous genetic material.

A typical plant promoter consists of specific elements. A basis is formed by the minimal promoter element, which enables transcription initiation often accompanied by a sequence, also denominated as the TATA-box, which serves as a binding place for transcription initiation factors. Presence of this TATA-box is a prerequisite for transcription. It is typically located 35 to 25 basepairs (bp) upstream of the transcription initiation site.
Other elements are known to be present generally in plant promoters. One often found common element in animals is the CAAT-box which maybe in plants would be represented as the AGGA-box, which is mostly located between position -70 and -100 (i.e. 100 to 70 bp upstream of the initiation site). However, little or no homology to these proposed boxes has been found (Elliston, K and Messing J. in: Kahl, G. (ed.), Architecture of Eukaryotic Genes, VCH, Weinheim, 1988, pp. 21-56).
Another part of the promoter are elements which are able to interact with multiple DNA-binding proteins. Known are G-box binding elements which are based on the hexanucleotide CACGTG motif. These elements have been shown to be able to interact with bZIP proteins which bind as dimers (Johnson & McKnight, Ann. Rev. Biochem, 58, 799-839, 1989).
Other G-box related motifs, such as the Iwt and PA motifs have been described (WO 94/12015).
These motifs have been shown to be involved in tissue-specific expression in plants. For instance, presence of Iwt tetramers confer

embryo-specific expression, while PA tetramers confer high level root expression, low-level leaf expression and no seed expression. Similarly, GT-1 like binding sites (grouped on basis of a moderate consensus sequence $GGT^{A}/_{T}A$) are described. Such a binding site is found far upstream the promoter region of the *Arabidopsis* plastocyanin promoter and seems to be involved in activation of transcription during light periods (Fisscher, U. *et al.*, Plant Mol. Biol. 26, 873-886, 1994).

Another sequence-related phenomenon which is found often in plant promoters is the presence of sequences which enable the formation of Z-DNA. Z-DNA is DNA folded in a left-handed helix which is caused by repeats of dinucleotides GC or AC. It is believed that folding in a Z-form influences the availability of the DNA for approach by polymerase molecules, thus inhibiting the transcription rate.

One of the early and most important inventions in the field of plant protein expression is the use of (plant) viral and *Agrobacterium* promoters that provide a powerful and constitutive expression of heterologous genes in transgenic plants. Several of these promoters have been used very intensively in plant genetic research and still are the promoter of choice for rapid, simple and low-risk expression studies. The most famous are the 35S and 19S promoter from Cauliflower Mosaic Virus (CaMV), which was already found to be practically useful in 1984 (EP 0 131 623), the promoters which can be found in *Agrobacterium* like the nopaline synthase (nos), mannopine synthase (mas) and octopine synthase (ocs) promoters (EP 0 122 791, EP 0 126 546, EP 0 145 338). A plant-derived promoter with similar characteristics is the ubiquitin promoter (EP 0 342 926).

In time, several attempts have been made to increase the level of expression of these promoters. Examples for this are the double enhanced 35S promoter (EP XXXX) and, more recently, the superpromoter, which couples parts of the *Agrobacterium* promoters (WO XXXX).

However, there is still need for new constitutive promoters which give a high level of expression.

SUMMARY OF THE INVENTION

The invention provides for a new constitutive plant promoter, characterized in that it comprises 1) a minimal promoter and 2) transcription-activating elements from a set of promoters, which elements direct a complementary pattern and level of transcription in a plant.

More specifically, this constitutive plant promoter is a promoter in which each of the transcription-activating elements do not exhibit an absolute tissue-specificity, but mediate transcriptional activation in most plant parts at a level of >1% of the level reached in the part of the plant in which transcription is most active. An example of such promoterpairs is a set of promoters in which one is most active in green parts of the plant, while the other promoter is most active in underground parts of the plant. More specifically the new promoter is a combination of the ferrodoxine and the RolD promoter. Preferably in this construct the minimal promoter element is derived from the ferredoxin promoter and the ferredoxin promoter is derived from *Arabidopsis thaliana*.

Also part of the invention is a constitutive plant promoter which is a combination of the plastocyanine and the S-adenosyl-methionine-1 promoter, whereby preferably the minimal promoter element is derived from the S-adenosyl-methionine-1 promoter and both the plastocyanine promoter and the S-adenosyl-methionine-1 promoter are derived from *Arabidopsis thaliana*.

Further part of the invention are chimaeric gene constructs for the expression of genes in plants comprising the above disclosed promoters.-

DESCRIPTION OF THE FIGURES

Figure 1: Schematic representation of pMOG410 and pMOG1059

Figure 2: Distribution of GUS expression of potato lines transformed with the constructs pMOG1059 en pMOG410. GUS staining was judged visually and classes of expression, relative to the highest GUS expression measured in our lab (set at 5). A value of zero indicates no visible expression.

Figure 3: Graphic representation of the average expression of GUS enzyme in primary transformants of tomato, oilseed rape and potato. GUS expression was determined visually and compared to a high level expressing 35S GUS transgenic tobacco plant ranking a score of 4. Standard deviation of the measured values are indicated on each of the bars.

DETAILED DESCRIPTION OF THE INVENTION

For the purpose of this specification the following definitions are valid:

A **promoter** consists of an RNA polymerase binding site on the DNA, forming a functional transcription initiation start site. A promoter usually consists of at least a TATA box and possibly of other sequences surrounding the transcription initiation site (initiator) and can either be used isolated (minimal promoter) or linked to binding sites of transcription-activating elements, silencers or enhancers) that may enhance or reduce transcription initiation rates, and which may function respective of developmental stage or external- or internal stimuli.

The initiation site is the position surrounding the first nucleotide which is part of the transcribed sequence, which is also defined as position +1. With respect to this site all other sequences of the gene and its controlling regions are numbered. Downstream sequences (i.e. further protein encoding sequences in the 3' direction) are denominated positive, while upstream sequences (mostly of the controlling regions in the 5' direction) are denominated negative.

A **minimal promoter** is a promoter consisting only of all basal elements needed for transcription initiation, such as a TATA-box and/or initiator.

An **enhancer** is a DNA-element which, when present in the neighbourhood of a promoter is able to increase the transcription initiation rate.

A promoter is **constitutive** when it is able to express the gene that it controls in all or nearly all of the plant tissues during all or nearly all developmental stages of the plant.

Specific expression is the expression of gene products which is limited to one or a few plant tissues (spatial limitation) and/or to one or a few plant developmental stages (temporal limitation).

The **expression pattern** of a promoter is the pattern of expression levels which shows where and in what developmental stage transcription is initiated by said promoter.

Expression patterns of a set of promoters are said to be complementary when the expression pattern of one promoter shows little overlap with the expression pattern of the other promoter.

The level of expression of a promoter can be determined by measuring the 'steady state' concentration of a standard transcribed reporter mRNA. This measurement is indirect since the concentration of the reporter mRNA is dependent not only on its synthesis rate, but also on the rate with which the mRNA is degraded. Therefore the steady state level is the product of synthesis rates and degradation rates. The rate of degradation can however be considered to proceed at a fixed rate, and thus this value can serve as a measure of synthesis rates. When promoters are compared in this way hybridisation S1-RNAse analysis, Northern blots, and competitive RT-PCR analysis are available to those skilled in the art. This list of techniques in no way represents all available techniques, but rather describes commonly used procedures used to analyse transcription activity and expression levels of mRNA.

One of the technical difficulties encountered in such an analysis is that the qualitatively best results can only be obtained by fusing transcriptional activating parts to the reporter RNA molecule, in such a way that only reporter sequences are transcribed. This requires the exact determination of the RNA synthesis start, and joining at that point the sequences of the reporter mRNA.

This is important for a number of reasons. First, the analysis of transcripion start points in practically all promoters has revealed that there is usually no single base at which transcription starts, but rather a more or less clustered set of initiation sites, each of which accounts for some start points of the mRNA. Since this

distribution varies from promoter to promoter the sequences of the reporter mRNA in each of the populations would differ from each other. Since each mRNA species is more or less prone to degradation, no single degradation rate can be expected for different reporter mRNAs. Secondly, it has been shown for various eukaryotic promoter sequences that the sequence surrounding the initiation site ('initiator') plays an important role in determining the level of RNA expression directed by that specific promoter. This includes also part of the transcribed sequences. The direct fusion of promoter to reporter sequences would therefore lead to much suboptimal levels of transcription.

Leaving in these transcribed sequences does allow determining the transcription rates, but potentially alters the stability of the reporter mRNA and influences translation initiation rates of an eventual coupled protein.

The role of this analysis, however, is the determination of the relative level of constitutive expression of a heterlogous protein, as is the most frequent used application of in biotechnology. Therefore the most important parameter is the ability of the tested sequences to drive high level expression of a heterologous reporter protein.

This would involve coupling the coding sequences of a reporter protein to the transcription activating part, promoter and 5' untranslated sequence of the gene which is tested for its properties. In this way a complex set of effects (combining transcription rates, mRNA stability (and thus degradation rates of the mRNA) and translational initiation rates) is reduced to one value that is a very useful value for determining usefulness of the tested gene elements in biotechnological applications.
There is no current words or phrase to describe this value. In the course of this application next to the term '**expression value**' the terms 'expression level' and 'transcriptional activity' are used. We realize that this may cause some confusion. In all cases we do indicate with these and related terms the value just mentioned.
A commonly used procedure to analyse expression patterns and levels is then through determination of the 'steady state' level of protein accumulation in a cell. Commonly used candidates for the reporter gene, known to those skilled in the art are ß-glucuronidase (GUS),

Chloramphenicol Acetyl Transferase (CAT) and proteins with fluorescent properties, such as Green Fluorescent Protein (GFP) from *Aequora victoria*. In principle, however, many more proteins are suitable for this purpose, provided the protein does not interfere with essential plant functions. For quantification and determination of localization a number of tools are suited. Detection systems can readily be created or are available which are based on e.g. immunochemical, enzymatic, fluorescent detection and quantification. Protein levels can be determined in plant tissue extracts or in intact tissue using in situ analysis of protein expression.

Generally, individual transformed lines with one chimeric promoter-reporter construct will vary in their levels of expression of the reporter gene. Also observed frequently is the phenomenon that such transformants do not express any detectable product (RNA or protein). The variability in expression is commonly ascribed to 'position effects' although the molecular mechanisms underlying this inactivity are usually not clear.

The term **average expression** is used here as the average level of expression found in all lines that do express detectable amounts of reporter gene, so leaving out of the analysis plants that do not express any detectable reporter mRNA or -protein.

Root expression level indicates the expression level found in protein extracts of complete plant roots. Likewise, 'leaf-' and 'stem expression levels' are determined using whole extracts from leaves and stems. It is acknowledged however, that within each of the plant parts just described, cells with variable functions may exist, in which promoter activity may vary.

For the promoter decsribed in this application the expression levels in large plant parts, containing cells with various functions, are measured. However, more detailed analyses may contribute to construction of a promoter that is even 'more constitutive' taking into account that more celltypes within a plant part are taken into account.

As a standard for judging expression levels the 35S promoter of the Cauliflower Mosaic Virus is a convenient and widespread used standard. The average expression level of this promoter may be classified as medium high.

The invention shows that it is possible to combine elements from one promoter which are responsible for a specific expression with elements from another promoter which are responsible for a complementory expression pattern to form a promoter which - as a result - shows expression in the tissues and developmental stages which form part of the expression pattern of both promoters. It seems to be necessary, however, that both promoters have a low expression value in the tissues and developmental stages which are specific for the other promoter. It has been established that, for being suitable, the transcriptional activity in the plant parts where expression is low should be at least >0.01% of the level of transcription which is reached in the plant parts where transcriptional activity is high.

This limits the availability of promoters and promoter elements from which to build a new constitutive promoter. Suitable promoter-pairs which fulfill the above mentioned criteria are:

- the ferredoxin promoter in combination with the rolD promoter
- the S-adenosyl methionine promoter in combination with the plastocyanin promoter

Other promoter-pairs which are complementary and which show at least some expression in the tissues and developmental stages which are specific for the other promoter can also be applied.

Delineation of promoter and/or enhancer parts needed.

Whereas transcription-regulating elements, especially in eukaryotes, may be present at large distances from the promoter/transcription initiation site, and located both downstream or upstream of the initiation site, many plant genes have most of their regulatory elements in the area directly upstream of the promoter. In order to identify the main transcription-activating elements of promoters it is common procedure to link parts of the non-transcribed areas upstream (and downstream) of the promoter and to analyse the ability of each of the truncated DNA elements to direct expression of a reporter gene. For delineation of more promoter-proximal sequences involved in transcription regulation, fragments of the enhancer sequences are most commonly coupled to a promoter, which may be derived from the gene of which transcription regulation is studied. Alternatively, a heterologous promoter can be used such as the sequences of the 35S

promoter from -46 to +4, relative to the transcription start, which is functionally coupled to a reporter gene as described above.
In this way it is possible to delineate the transcription activating elements of most genes, a process that is well-known to those skilled in the art.
A large number of transcription regulatory elements of genes have been analysed in such a manner, and data relevant for this analysis are directly available to those skilled in the art through scientific publications.
Transcription activating elements that on average can direct expression to approximately the average level of the 35S promoter (at least 50% of this level) in at least some of the plant parts, and that are also capable of directing at least 0.5% (of the 35S level) transcription in other plant parts are then selected for further use.

The minimal promoter element is typically derived from one of the promoters of the promoter-pair, although not necessarily. It can be envisaged that such a minimal element is derived from a third promoter or even made synthetically.

Based on the results of the analysis described above, transcription activating parts with complimentary activities are selected. That is, transcription activating DNA fragments that direct high level root expression and with lower leaf and stem expression levels, are combined with elements that direct expression mainly in the leaf and stem, but lower in the root. Other combinations of complimentory transcription activating parts are obvious. Preferentially, the level of expression in the parts where expression is lowest does not fall below 1% of the level obtained in the highest part. More preferred is the situation where the relation between lowest expression and highest expression between plant parts is larger than 5%.

This coupling can most easily be done by known genetic engineering techniques. The gene which has to be expressed by the new constutive promoter can be cloned behind the promoter. It is adviseable to build in a unique NcoI-cloning site at the linkage of the 5' untranslated sequence attached to the promoter to allow precise junction of the open reading frame (ORF) and the 3' end of the promoter in which the gene of interest can be inserted.

The ferredoxin-rolD pair.

One of the preferred combinations of the present invention is a constitutive plant promoter comprising elements of both the ferredoxin promoter and the rolD-promoter. Preferably the ferredoxin promoter is obtained from *Arabidopsis thaliana* where it drives the ferredoxin A gene, a gene which is involved in the photosynthesis. The expression of this gene and the responsiveness of its promoter to light has been reported (Vorst, O. et al., Plant Mol. Biol. 14, 491-499, 1990; Vorst, O. et al., The Plant J. 3(6), 793-803, 1993; Dickey, L.F. et al., The Plant Cell 6, 1171-1176, 1994). Since the ferredoxin gene is involved in photosynthesis the promoter is active in green tissue. mRNA levels were shown to be high in chloroplast-containing organs such as stem, leaves and bracts, but also in young growing tissues, such as whole flowers and seedlings. The promoter sequence contains both a G-box and an I-box containing region. Also a potential Z-folding DNA sequence is found at position -182.

The rolD promoter is reported to be a root-specific promoter obtainable from *Agrobacterium rhizogenes*. Although the source organism is a bacterium, the promoter is very suitable for expression in plants because the bacterium is a phytopathogen which causes hairy-root disease in plants. For that purpose it transfers DNA to the plant amongst which the rolD gene is responsible for root elongation. To be expressable in plants this gene needed a strong promoter, the rolD promoter. GUS-studies have shown that expression under control of the rolD-promoter yields mainly root-specificity (Leach, F. and Aoyagi, K., Plant Sci. 79, 69-76, 1991). Also expression in leaves was observed.

A combination of the ferredoxin and the rolD promoter can be obtained in two ways, depending on from which promoter the minimal promoter element and 5' untranslated sequences will be taken. In our examples we have used the minimal promoter element from the ferredoxin promoter, but deriving it from the rolD promoter is equally well possible.

The S-adenosyl-methonine synthetase and plastocyanine pair.

Another favourable constitutive promoter can be obtained from a combination of the S-adenosyl-methionine synthetase (SAM) promoter and specific parts of the plastocyanin promoter. Preferably, both promoters are obtained from *Arabidopsis thaliana*.

The SAM promoter regulates the expression of S-adenosyl-methionine synthetase, which is an enzyme active in the synthesis of polyamines and ethylene. Promoter studies showed a strong expression in vascular tissues, in callus, sclerenchyma and in root cortex (Peleman, J. et al., The Plant Cell 1, 81-93, 1989) which was reasoned to be due to the involvement of the enzyme in lignification.

The plastocyanin promoter, like the ferredoxin promoter, is also a promoter which is active in the photosynthetic pathway. mRNA levels are high in green, chloroplast-containing structures, such as leaves, cauline leaves, stem and whole seedling. Also in flowers the promoter is very active. Little expression is detectable in silique, seed and root (Vorst, O. et al., The Plant J. 4(6), 933-945, 1993).

Other pairs of promoters.

The above given examples of promoter-pairs show in both cases the presence of a promoter which is active during photosynthesis. It is envisaged that other promoters which are regulating expression of a gene needed for photosynthetic activity may be suitable for a combination with either the rolD or the SAM promoter.

If one of the components is a promoter which is more or less specific for green parts, this automatically means that the other promoter of the pair should be predominantly expressed in the roots and other non-photosynthesizing organs..

However, the invention is not limited to the combination of a root-specific and a green part-specific promoter. All promoter combinations provided that the expression patterns of the individual promoters are complementary can be used.

It is also possible that the elements from which the new constitutive promoter is composed are derived from a set with more than two promoters. The above discussed complementarity should then also exist.

EXPERIMENTAL PART

Example 1

Cloning of the chimeric Fd-rolD promoter:

A 512 bp *Arabidopsis thaliana* ferredoxin promoter fragment (O. Vorst et al., 1990, PMB 14, 491-499.) ranging from position -512 to +4 (relative to the ATG startcodon of the ferredoxin Open Reading Frame) was isolated by digestion with HincII and NcoI. This fragment contains most of the transcriptional regulatory sequences of the ferredoxin promoter, the promoter sequences and leader of the ferredoxin transcript. An XbaI site was introduced, for cloning reasons, at positions -5 to -10 relative to the ATG. This changes the original sequence of the clone at this point from ACAAAA to TCTAGA.

Part of the *Agrobacterium rhizogenes* rolD upstream sequences (Leach et al., 1991 Plant Sci. 79, 69-76) were fused to the ferredoxin promotersequences described above. A HindIII-RsaI fragment, comprising nucleotides -385 to -86 relative to the initiation codon was cloned next to the ferredoxin fragment, joining the RsaI sites of the latter with the HincII site of the former.

This chimeric element, containing the promoter and some of the activating sequences of the ferredoxin gene, and upstream activating sequences of the rolD gene was used in subsequent studies as to its transcription-stimulating properties.

Example 2

GUS-fusions

The Fd-rolD chimeric promoter/activator was coupled to the GUS gene, engineered to contain an intron. The NcoI restriction site on the ATG start codon was used to join the promoter to the Open Reading Frame (ORF) of the GUS gene. Subsequently this construct (pMOG1059) was used in transformation experiments with various plants. As a control a 35S CaMV promoter-GUS construct was used. This is construct pMOG410. A schematic representation of both constructs is found in figure 1.

Example 3

Expression levels and patterns of promoter activity during early stages of plant transformation

First, *Arabidopsis thaliana* transformants were made with both constructs and GUS expression was followed in tme during the transformation procedure.

GUS expression levels were determined visually, on a scale of 0 to 5, where 0 is no detectable expression and 5 is the highest level of GUS observed in a transgenic plant, and is a rare tobacco 35S-GUS-transgenic line 96306. Samples from this plant were included in all experiments for internal reference.

In table 1 the relative GUS expression in *Arabidopsis thaliana* explants is indicated, at several times after *Agrobacterium tumefaciens* cocultivation (DAC; days after cocultivation)

Table 1. relative GUS activity of Arabidopsis root explants.

Construct : Time of assay	pMOG1059	pMOG410
DAC 0	2	3
DAC 2	3	3
DAC 5	3	3
DAC 7	4	3
DAC 9	4	3
DAC 12	4	3

As can be seen from this comparison, GUS expression driven by the chimeric promoter starts slightly later after cocultivation but from day 7 on, exceeds the level of expression obtained with the reference 35S promoter.

Very similar data were obtained when *Brassica napus* explants were scored for GUS expression. At day 5 after co-cultivation the 35S promoter is slightly higher, but the situation is reversed on day 20 after co-cultivation. Also for tomato similar data were obtained. Here even at the earliest stage of analysis expression of pMOG1059-transgenics exceeded that of pMOG410 transgenics.

Example 4

Expression levels and patterns in *in vitro* grown plants

When plants are grown up further, differences between these promoters become ever clearer. Leaf samples of fully regenerated plants were analysed for GUS expression. Averages were obtained from 11-48 plants, dependent on the construct.

For *Arabidopsis thaliana* that was grown in vitro only, no significant difference was observed between GUS expression in pMOG1059 and pMOG410-transgenics.

Table 2. Average relative GUS activity of leaf samples of all tested crops.

Construct :	pMOG1059	pMOG410
Crop :		
Potato	4.0	2.1
Brassica napus	3.7	2.8
Arabidopsis	4.0	4.1
Tomato	in progress	in progress

What is also clear from the data presented in figure 2 that a significant number of 35S-GUS transgenic lines (appr. 50% was found repeatedly in our experiments) do not express GUS to a level that it is visible. So not only maximum and average expression are higher in the Fd-rolD-GUS transgenics, also the frequency with which transgenic plants do express GUS is strongly enhanced, in about 50 transgenic potato plants carrying the Fd-rolD-GUS construct, we have found no weak expressor, suggesting a reliable high expression in at least 98% of the lines made.

Example 5

Comparison of promoter performance in various crops

Constructs pMOG410 (35S-GUS) and pMOG1059 (Fd-rolD-GUS) were also introduced into oilseed rape and tomato for a further comparison of promoter performance. Also the data for potato are included here. As shown in Figure 3A, in tomato the overall level of expression of the Fd-rolD promoter is higher both at the latest stage of *in vitro* growth as well as in leaves of 4 and 7 week old plants. Also in stems of 7 weeks this holds true, however, for roots, an average weaker expression is observed with the Fd-rolD promoter than for the 35S promoter.

Also in oilseed rape and potato, similar results are obtained, with the notable exception that in potato roots the level of expression by the Fd-rolD promoter exceeds that of the 35S promoter. As shown in figures 3B and 3C both the average expression of the Fd-rolD promoter

is higher and also the variation in expression is significantly lower. In conclusion we can say we have created a promoter that withstands the comparison with the 35S promoter easily in three major crops.

Example 6

Expression of nptII transgene.

In order to also check usability of the Fd-rolD promoter for other purposes, the promoter was linked to the nptII gene, of which expression of the corresponding gene product confers resistance in plants to the antibiotic kanamycin. This element was placed between the left and right borders of the T-DNA allowing *Agrobacterium tumefaciens*-mediated transfer to plants. As a control, similar constructs in which the expression of the nptII gene was under control of the nos promoter were used.

The resistance to kanamycin in transgenic potato plants is manifested by the development of transgenic calli and shoots during a standard transformation procedure, in which kanamycin is used in the culture medium.

On average, for the constructs with the nos-nptII selction cassette, the transformation frequency for potato is 45%, for constructs with the Fd-rolD-nptII selection cassette the frequency is on average 61%. While we do not know at this moment how relevant the increase in transformation frequency is for this construct, it indicates that the Fd-rolD promoter is at least as suitable for driving a heterologous gene such as nptII, as commonly used constitutive promoters such as nos.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(ii) TITLE OF INVENTION: New constitutive plant promoters

(iii) NUMBER OF SEQUENCES: 5

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(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 878 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAAAAGAAGA GAGAAGTGAG AATCGTCTCT GTTTTCAGAA ACTCTGAAAA ACGTTTATGA 60

CCACGTGTTT TCCAGAAATG ATTCATTTTA TTCTTTTTAT TAAAATTTAA TACTTTATCT 120

AAATTCAATT AAAATAAGCA ATATTTTATT CATGAGAAAT TCTTTTTTGA GAATCAACCG 180

ATGTAGATGG TCTCATACTC TACTCTGTTG ATTGTGTTTA AGTTTCTGAG GATTTTTCTA 240

CTTTCCGACG TTATGCCAAG AGGCTGGTCT TCACTAGAAA ACTACTTCCA CCCAATTCAA 300

GCAAGTATGA CCTCTTCTCC CCACCATTTA TTCATGTACT GAAAGGCCAT TAGAAGTTGA 360

CTGAAGTGTG AAGGTGGAGA TTATGTATTC ACTTGTTGAT TTGGTATACA TTCTATGTAA 420

GGTTCAATTA TTTACGTTAT ATAATTATAA TGGAGTAATT TACAGTAATT GGGTTAAAAT 480

GGTTTGATTC GGTCAGGTTG ATACGGTTTG GAAGTTAAAC CCGGCCTAGA TATGATGTTA 540

CAACCAGTCC ACATCTTTTA TGATTTTAGT GGAACAAACG AAGAGTTATT TAGACGATAC 600

AAACAAGGTC CGAATAAGTG TGAGCTGTCC CAAGTAAGAC CACGTAATAC TCACCTCAAC 660

AAGATAGTGT TCTTAAAGTG TGTCAAACAC AATCACACAC ACACAAATCA TAAAACACAA 720

AGACGATAAT CCATCGATCC ACAGAATAGA CGCCACGTGG TAGATAGGAT TCTCACTAAA 780

AAGTTCTCAC CTTTTAATCT TTCTCCACGC CATTTCCACA AGCCATAATC CTCAAAAATC 840

TCAACTTTAT CTCCCAAAAC ACAAATCTAG AAACCATG 878

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCCACTACAA TGAATTTGTT CGTGAACTAT TAGTTGCGGG CCTTGGCATC CGACTACCTC 60

TGCGGCAATA TTATATTCCC TGGGCCCACC GTGAACCCAA TTTCGCCTAT TTATTCATTA 120

CCCCCATTAA CATTGAAGTA GTCATGATGG GCCTGCAGCA CGTTGGTGAG GCTGGCACAA 180

CTCATCCATA TACTTTCTGA CCGGATCGGC ACATTATTGT AGAAAACGCG GACCCACAGC 240

GCACTTTCCA AAGCGGTGCC GCGTCAGAAT GCGCTGGCAG AAAAAAATTA ATCCAAAAGT 300

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACTGAAGTG TGAAGGTGGA GATTATGTAT TCACTTGTTG ATTTGGTATA CATTCTATGT 60

AAGGTTCAAT TATTTACGTT ATATAATTAT AATGGAGTAA TTTACAGTAA TTGGGTTAAA 120

ATGGTTTGAT TCGGTCAGGT TGATACGGTT TGGAAGTTAA ACCCGGCCTA GATATGATGT 180

TACAACCAGT CCACATCTTT TATGATTTTA GTGGAACAAA CGAAGAGTTA TTTAGACGAT 240

ACAAACAAGG TCCGAATAAG TGTGAGCTGT CCCAAGTAAG ACCACGTAAT ACTCACCTCA 300

ACAAGATAGT GTTCTTAAAG TGTGTCAAAC ACAATCACAC ACACACAAAT CATAAAACAC 360

AAAGACGATA ATCCATCGAT CCACAGAATA GACGCCACGT GGTAGATAGG ATTCTCACTA 420

AAAAGTTCTC ACCTTTTAAT CTTTCTCCAC GCCATTTCCA CAAGCCATAA TCCTCAAAAA 480

TCTCAACTTT ATCTCCCAAA ACACAAATCT AGAAACCATG 520

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 753 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTGCAGCGAT TTCATTTTAG ATTCTCAAAA ATATTCTCCG ATGTGTGGGA TTTGAGTAGA 60

TTTTGTGTGT TGGCATGATT CGAATAGTAT GCAAGATTGT TGGAGTATTA GAGTTGAATT 120

GGTATTTTAG CTTTAGTTTT AATGAGTCTT TAAGTTGTTT TTCAAGTTTG AATAAGCTCC 180

TGGTTTGTAG GAGTCAAGTA GTAGTGGTCC TAGTCATTAG TTTACTTCCG CAAGTCTCTC 240

ATTGTTTAGT TGTAGGAGAC TTAGTCTTCA TGTCATTGGC TATTTAAGGC CCACCAATTC 300

TCAGCAATAT AGTATCGTGG TATTTGAGAA AACCTGCAAC TTTATTTTCT ACATGTCTGC 360

AACTTTATTT TCTACAAAGA TTTTTGAGAT TTTGAATTCG TTCATGTATG TATGTGTGAT 420

TGTAGCTTGA TATGATTTAA TCTGTTAGTT AAATGTGCAC AGACAATAAG TAACATAAGA 480

AGCGAGTCAC TAAGGATAAG AGTCAACTTG TTTTGCTGAA AAGATATCAC TTATGATTTT 540

CGAATCATTT TAGCTTTTTG TCACTTGAGC TTAATGATTC TTCTGAAATT CGATTCTTTG 600

TTTTGTTTGT ATCACATTCT TTAGAATTTG GAATCTAAGA AAAGCTTTCA GGATATGGTG 660

AAACTATTCT TTTAAGATAG CATGATGCTT CTTTAATGAT TATCTACAGT GACTAAGTCA 720

GTTTGTTTTG TTCTATTCTT TGTAGCACCA TGG 753

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 876 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATCATAATAC TCATCCTCCT TCTCAAGGTT CGTACGTATT ATCAATATCT AGTATATACT 60

TGTCTTTGTT CTATGCTTTA TATCATCATT TTATGACAAA AAATGATTAA GGTCTTAGTT 120

AATGATTATG TATATGTGAA ACTTATATTT AGGGGCACAA TTTAATTTCG TATGATAATT 180

GTCTAGTTAG CTTTTATGTAC TTATCATAAA AACCTTAGTG TTTATCGCAA TACTTTTCAA 240

ATATAGTGTA GAATCATAAT GGTCCCACTG TCATTATGTT TGATGCAAAT CTATTTGGAT 300

TTTGTTGGAT AATAAACCGA TGACGTGGAC CAGACCAGTA GCTATAAGAT TTGGTTCACA 360

TAGAAATTTT TTATAAGATA ATGTATCTAG GTTTGCTTAT GATTATACAT GTGATATTTA 420

ATACATGGCA CAGGTTCGTC GAGTTTCACA GCCATAGGTA CAATAGAAGG CAAATTCGAT 480

TGTGGTTATC TGGTAAAAGT TAAGTTGGGC TCAGAGATTC TTAACGGCGT TCTTTATCAT 540

TCGGCCCAGC CCGGCCCATC ATCATCTCCA ACCGCTGTTC TAAACAATGC CGTTGTACCT 600

TATGTTGAAA CTGGGAGGAG ACGGCGTCGT TTAGGTAAAA GACGAAGAAG CAGACGCAGA 660

GAAGATCCGA ATTACCCGAA ACCGAACCGG AGCGGTTACA ATTTCTTCTT TGCTGAGAAA 720

CATTGCAAGC TCAAATCACT TTATCCCAAC AAGGAGAGAG AGTTTACGAA ACTTATCGGA 780

GAATCGTGGA GCAATCTCTC TACCGAAGAA CGAATGGTAA CAAATTATCT TTTAAACCGT 840

TACCGATTTA GTGATGAAAT TAGATTTGTA GTAAAT 876

CLAIMS

1. Constitutive plant promoter, characterized in that it comprises a minimal promoter and transcription-activating elements from a set of promoters, which elements have a complementary pattern and level of transcription in a plant.

2. Constitutive plant promoter according to claim 1, characterized in that each of the transcription-activating elements do not exhibit an absolute tissue-specificity, but mediate transcriptional activation in most plant parts at a level of ≥1% of the level reached in the part of the plant in which transcription is most active.

3. Constitutive plant promoter according to claim 1 or 2, characterized in that one promoter of the set of promoters is specifically active in green parts of the plant, while the other promoter is specifically active in underground parts of the plant.

4. Constitutive plant promoter according to claim 3, characterized in that it is a combination of the ferrodoxine and the RolD promoter.

5. Constitutive plant promoter of claim 4, characterized in that the minimal promoter element is derived from the ferredoxin promoter.

6. Constitutive plant promoter according to claim 4 or 5, characterized in that the ferredoxin promoter is derived from *Arabidopsis thaliana*.

7. Constitutive plant promoter according to claim 6, characterized in that it comprises the sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

8. Constitutive plant promoter according to claim 3, characterized in that it is a combination of the plastocyanine and the S-adenosyl-methionine-1 promoter.

9. Constitutive plant promoter according to claim 8, characterized in that the minimal promoter element is derived from the S-adenosyl-methionine-1 promoter.

10. Constitutive plant promoter according to claim 8 or 9, characterized in that the plastocyanine promoter is derived from *Arabidopsis thaliana*.

11. Constitutive plant promoter according to claim 8, 9 or 10, characterized in that the S-adenosyl-methionine-1 promoter is derived from *Arabidopsis thaliana*.

12. Constitutive plant promoter according to claim 11, characterized in that it comprises the sequences of SEQ ID NO:4 and SEQ ID NO:5.

13. Chimaeric gene construct for the expression of genes in plants comprising the promoter of any of claims 1-12.

ABSTRACT

The invention describes new constitutive promoters build from elements from a set of promoters which have a complementary expression pattern.


pMOG410
LB
nos-nptII
PI-II
GUS + intron
RB 35S CaMV
pMOG1059
LB
nos-nptII
PI-II
GUS + intron
RB Fd-rolD chimeric

Fig. 1

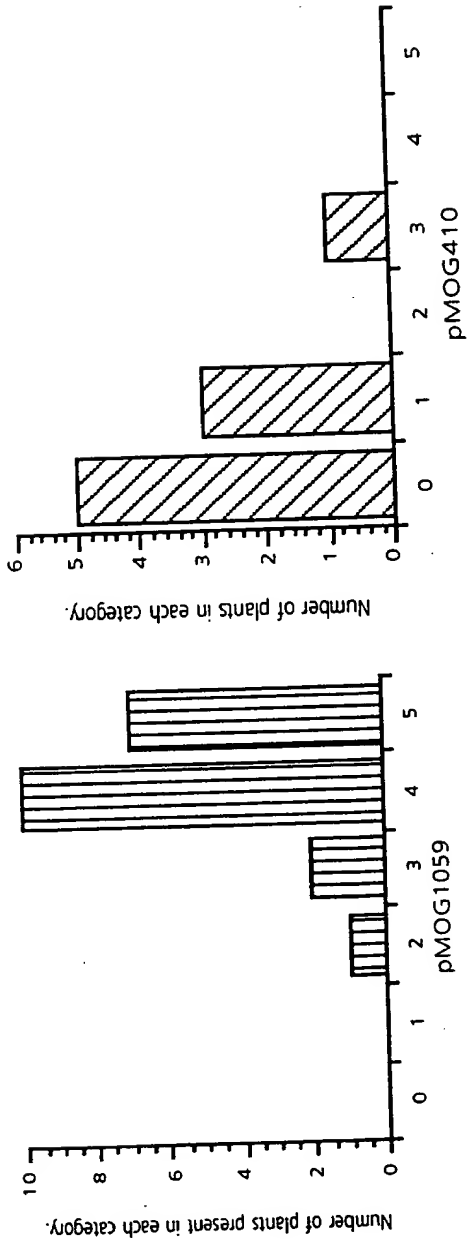
Number of plants present in each category.
0
2
4
6
8
10
0
1
2
3
4
5
pMOG1059
Number of plants in each category.
0
1
2
3
4
5
6
0
1
2
3
4
5
pMOG410

Fig. 2

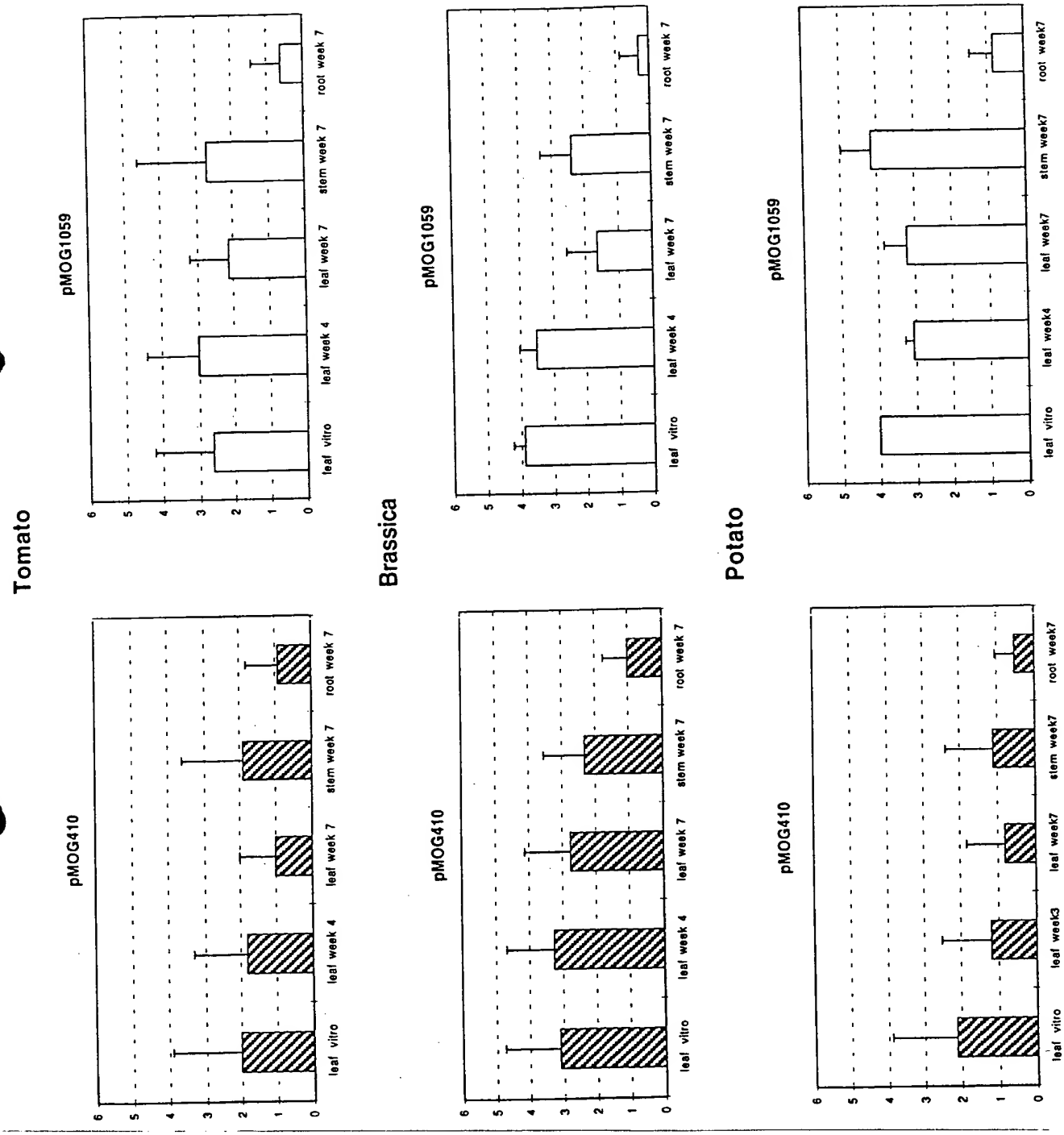
Tomato
Brassica
Potato
pMOG1059
pMOG410
leaf vitro
leaf week 4
leaf week 7
stem week 7
root week 7
leaf week3
leaf week4
leaf week7
stem week7
root week7
0
1
2
3
4
5
6

Fig. 3